REMARKS/ARGUMENTS

Status of the Application

In the Non-Final Office Action, claims 33-34 and 52-57 were objected to and claims 31-34 and 49-57 were rejected. In the present response, claims 33-34 and 49-57 were canceled without prejudice. Applicant reserves the right to pursue these claims in any application derived from the present application. Thus, claims 31-32 are pending. No new matter was added.

Objections Under 37 C.F.R. § 1.75(c)

Claims 33-34 and 52-57 were objected to under 37 C.F.R. § 1.75(c) as being of improper dependent form for failing to further limit the subject matter of a previous claim. Because claims 33-34 and 52-57 have been canceled, these rejections are moot.

Rejections Under 35 U.S.C. § 112, 1st Paragraph

Claims 33-34 and 50-57 were rejected under 35 U.S.C. § 112, 1st Paragraph, as failing to comply with the written description requirement. In the Office Action, the Examiner termed this rejection "A New Matter Rejection". Because claims 33-34 and 52-57 have been canceled, these objections are moot.

Claims 31-34 and 52-57 were rejected under 35 U.S.C. § 112, 1st Paragraph, as failing to comply with the enablement requirement. Because claims 33-34 and 52-57 have been canceled, these rejections are moot. Applicant respectfully traverses the enablement rejections of claims 31-32.

In determining whether the amount of experimentation needed is undue, the Examiner correctly cited *In re Wands*, 858 F.2d 731 (Fed. Cir. 1988), for the non-exhaustive factors listed therein. Applicant disagrees, however, with the Examiner's conclusions regarding the predictability of the ghrelin art and the quantity of experimentation needed to make or use the claim 31 and 32 inventions.

Level of Predictability in the Art

The Examiner argues that "no studies have been performed that clearly demonstrate the predictive value of a diagnostic assay for diabetes or obesity." Applicant notes, however, that in the first five years after the discovery of ghrelin and prior to the present application's filing date (that is, 1999-2003), 571 peer-reviewed papers[1] were published on ghrelin, including structure related papers, animal studies, and human clinical studies from different groups in different locations in the world, and 1640 ghrelin peer-reviewed papers have been published to date. Because similar results were generated from most of the groups in mice, rats, beagles, pigs, or humans using different modes of administration (intravenous, subcutaneous, intra peritoneal, intra cerebral, et cetera), it would be a matter of *routine experimentation* by one of ordinary skill in the art to reproduce these results and to test similar peptides, for example ghrelin splice variant 2, in comparison to ghrelin. The following peer-reviewed papers, all attached hereto, are merely a sampling of those papers published prior to Applicant's filing date that demonstrate ghrelin's relationship to obesity and diabetes: Tschop *et al.*, Nature 407:908-13 (2000); Wren *et al.*, Endocrinology 141:4325-28 (2000); Kamegai *et al.*, Endocrinology 141:4797-4800 (2000); Nakazato *et al.*, Nature 409:194-98 (2001); Asakawa *et al.*, Gastroenterology 120:337-45 (2001); Shintani *et al.*, Diabetes 50:227-32 (2001); Arvat *et al.*, J. Clin. Endocrinol. Metab. 86:1169-74 (2001); Kojima *et al.*, Trends Endocrinol. Metab. 12:118-22 (2001); Wren *et al.*, Diabetes 50:2540-47 (2001); Wren *et al.*, J. Clin. Endocrinol. Metab. 86:5992-95 (2001); Lawrence *et al.*, Endocrinology 143:155-62 (2002). Additional references are available upon request.

The usefulness of ghrelin for the treatment of obesity, diabetes, and/or cachexia is noted in many papers. *See, e.g.,* Root & Root, Curr. Drug Targets Immune Endocr. Metabol. Disord. 2:27-52 (2002). Muccioli *et al.* recognized, as noted in their abstract and conclusions, that "GHS receptor agonists or antagonists acting on appetite could represent new drug intervention for eating disorder."[2] Broglio *et al.* conclude at page 158 that "[t]he stimulatory effect of ghrelin and synthetic GHS analogs acting as agonists or antagonists to appetite

[1] NCBI Pubmed search, http://www.ncbi.nih.gov/entrez/query.fcgi.
[2] Eur. J. Pharmacol. 440:235-54 (2002).

could have potential in eating disorders and obesity."[3] Broglio *et al.* are also actively involved in developing non-acylated ghrelin as an anti-diabetic therapeutic (as presented at the 85th Annual Meeting of the Endocrine Society, Philadelphia, PA, June 18, 2003). Broglio *et al.* further concluded that non-acylated ghrelin may have a therapeutic potential in the treatment of insulin resistance.

Several physiological and pathophysiological conditions, including changes in body fat, food intake, and insulin resistance, are known to be associated with variations in plasma ghrelin concentrations. Circulating ghrelin levels have also been shown to be decreased in human obesity. For example, plasma ghrelin concentration was lower in obese Caucasians and Pima Indians (a population with a very high prevalence of obesity) compared with a lean control population (*see* Tschop *et al.*, Diabetes 50:707-09 (2001); Dhillo & Bloom, Curr. Opin. Pharmacol. 1:651-55 (2001)). It was also shown that low ghrelin concentrations in nonalcoholic fatty liver disease are related to insulin resistance (*see* Marchesini *et al.*, J. Clin. Endocrinol. Metab. 88:5674-79 (2003)). Based on this showing, ghrelin antibodies are used to detect deterioration in a patient's insulin sensitivity status and can be an early predictor for insulin resistance. Using antibodies for all of the splice variants can improve the diagnosis as a more accurate picture of plasma circulating hormones will be available.

The Examiner cites Muccioli *et al.*, Eur. J. Pharmacol. 440:235-54 (2002), for the notion that "administration of wild type ghrelin to humans has resulted in hyperglycemia followed by reduced insulin secretion," leading to the Examiner's conclusion that "[t]hese effects are opposite of what one would want to induce for the treatment of diabetes, which suggests ghrelin antagonism as a potential method of therapy." More recent work suggests the opposite. For example, Broglio *et al.* concluded that des-acyl ghrelin has an antagonistic effect on insulin secretion and glucose metabolism[4]; thus, des-acyl ghrelin can be used as a therapeutic agent to treat insulin resistance in diabetes. Furthermore, Asakawa *et al.* demonstrated that des-acyl ghrelin acts as an antagonist to acylated ghrelin and significantly reduces food consumption.[5]

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[3] Treat. Endocrinol. 2:153-63 (2003).
[4] 85th Annual Meeting of the Endocrine Society, Philadelphia, PA, June 18, 2003.
[5] Gut 54:18-24 (2005).

The Examiner also find support in Muccioli *et al.* for the conclusion that "ghrelin has been shown to have orexigenic activity in rodents, which cannot be reaptiulated in humans." Quite to the contrary, ghrelin orexigenic activity was recapitulated in humans by *many groups* via different administration routes and different doses. *See, e.g.,* Cummings *et al.*, Diabetes 50:1714-19 (2001); Tschop *et al.*, J. Endocrinol. Invest. 24:RC19-21 (2001); Wren *et al.*, J. Clin. Endocrinol. Metab. 86:5992-95 (2001); Cummings *et al.*, Arch. Surg. 138:389-96 (2003); Broglio *et al.*, J. Clin. Endocrinol. Metab. 86:5083-86 (2001); Aimaretti *et al.*, Clin. Endocrinol. 56:765-71 (2002); Arvat *et al.*, J. Clin. Endocrinol. Metab. 86: 1169-74 (2001); Enomoto *et al.*, Clin. Sci. 105:431-35 (2003); Tassone *et al.*, J. Clin. Endocrinol. Metab. 88:5478-83 (2003); Cummings *et al.*, 346:1623-30 (2003). Further, Root and Root teach that ghrelin induces hunger in humans.[6]

The Examiner also asserts, citing Root and Root, that the function of ghrelin's carboxy terminal region is uncharacterized and concludes that "the claims encompass peptides that have no art-recognized activity. Tolle *et al.*[7], Torsello *et al.*[8], and Muccioli *et al.*[9], however, teach that to achieve ghrelin peptide activity *in vivo* a longer peptide is needed. Thus, the carboxyl sequence of ghrelin is important for *in vivo* activity. In Applicant's studies, chronic treatment of 129 Strain mice with ghrelin splice variant 2 in the either the acylated form or the non-acylated form reduced total cholesterol and LDL levels, while wild-type ghrelin had the opposite effect (*see, e.g.,* Applicant's co-pending U.S. Provisional Application Serial No. 60/781,860). Because the difference between ghrelin splice variant 2 and wild-type ghrelin is in the carboxyl sequence and the cholesterol effect was demonstrated in both acylated and non-acylated forms of ghrelin splice variant 2, Applicant postulates that the carboxyl end of ghrelin splice variant 2 has an important activity that might help in treating obese patients that also suffer from high cholesterol.

In relation to anti-ghrelin splice variant 2 antibodies, developing antibodies to block or decrease the activity of a gene product is a well-known approach for treating conditions where the expression or high level expression of a gene

[6] Curr. Drug Targets Immune Endocr. Metabol. Disord. 2:27-52 (2002).
[7] Neuroendocrinology 73:54-61 (2001).
[8] Endocrinology 143:1968-71 (2002).
[9] Eur. J. Pharmacol. 440:235-54 (2002).

product can cause harm in specific conditions. For example, Enbrel® (anti-TNFα), developed by Immunex, blocks the inflammation-causing protein TNFα and is being used as an anti-inflammatory treatment for several indications (for example, rheumatoid arthritis and psoriatic arthritis; *see* http://www.embrel.com). Rituxan® (anti-CD20: human B-lymphocyte-restricted differentiation antigen, Bp35), developed by Genentech and Biogen Idec, blocks and destroys CD20-carrying B-Cells thus treating Non-Hodgkin's Lymphomas (*see* http://www.rituxan.com). Tysabri® (anti-α4 integrin), developed by Biogen Idec, is an anti-inflammatory antibody for the treatment of multiple sclerosis. This antibody blocks and destroys cells carrying the α4 integrin protein (*see* http://www.tysabri.com).

By 2003, the art had already accepted ghrelin's therapeutic potential and started the process of developing ghrelin as a therapeutic for diabetes and eating disorders. For example, Nakazato *et al.* showed that it is possible to block feeding using anti-ghrelin antibodies,[10] and Cytos is already in Phase I/II clinical trials in the development of an anti-ghrelin antibody. Asakawa *et al.*, in their abstract, conclude that

> [g]hrelin appears to be closely related to excess weight gain, adiposity, and insulin resistance, particulary under a high fat diet and in the dynamic stage. Gastric peptide ghrelin and GHS-R may be promising therapeutic targets not only for anorexia-cachexia but also for obesity and type 2 diabetes, which are becoming increasingly prevalent worldwide.[11]

First clinical trial results were submitted for publication in October 2003 and published in June 2004 (*see*, Neary *et al.*, J. Clin. Endocrinol. Metab. 89:2832-36 (2004)). Additionally, ghrelin diagnostic kits and commercial antibodies are currently available off-the-shelf.

The ghrelin art regarding use of the claimed peptides is not unpredictable, contrary to the Examiner's assertions.

Applicant's 132 declaration filed in connection with the Response to the August 25, 2005, Final Office Action further demonstrates the predictability of the ghrelin art. Applicant chose to reproduce the first ghrelin animal study, Tschop *et al.*, Nature 407:908-13 (2000), and, as can be seen from the 132 declaration,

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[10] Nature 409:194-98 (2001)
[11] Gut 52:947-52 (2003).

Applicant *did* reproduce Tschop *et al.*'s experiment and demonstrated that non-acylated ghrelin splice variant 2 induces weight loss and reduces food consumption in mice. Non-acylated ghrelin splice variant 2 is thus a potential therapeutic agent for obesity. Acylated ghrelin splice variant 2 induced weight gain and food consumption, making it a good target for antibody development and a good peptide therapeutic for those suffering from unintentional weight loss or cachexia (*see, e.g.,* Applicant's co-pending U.S. Provisional Application Serial No. 60/781,860).

Quantity of Experimentation Needed

Applicant's 132 declaration demonstrates, using *routine experimentation*, that des-acyl ghrelin splice variant 2 induces weight loss and decreased food consumption. The experiment was performed with 129 Strain mice (a standard mice strain) ordered from Harlan UK (but available from many animal houses world wide). Non-acylated ghrelin splice variant 2 and acylated ghrelin splice variant 2 were produced by Bachem Biosciences (King of Prussia, PA), based on ghrelin splice variant 2 amino acid sequence supplied by Applicant and disclosed in the present application. The variants were administered daily for a period of five days. Animal weight and food consumption were measured daily.[12] Weighing animals and measuring amounts of consumed food is a very simple and standard procedure. Further, no special animal models were used, and no special modes of administration or parameters were used. Thus, one of ordinary skill in the art can reproduce this experiment easily and routinely starting with just Applicant's claimed sequences.

As it turns out, non-acylated ghrelin splice variant 2 will indeed be used for the treatment of obesity. Applicant is in the early stages of preclinical development towards an Investigational New Drug Application ("IND") using ghrelin splice variant 2 to treat obesity. The development plan and study designs are based on well-characterized preclinical and clinical study requirements familiar to the FDA from previously developed anti-obesity products. One of ordinary skill in the art will be able to build a treatment procedure using non-acylated ghrelin splice variant 2 based on similar treatment procedures already

[12] Food consumption was measured by deducting the amount of food left in the animals' cages from the amount that was placed there each day.

approved by the FDA using peptides such as, for example, growth hormone, insulin, calcitonin, or erythropoietin. Applicant's treatment procedure currently is being developed for investigational drugs using ghrelin on the appropriate set of patients. Exemplary clinical study designs are available at http://www.clinicaltrials.gov/ct.

In relation to production of an anti-ghrelin splice variant 2 antibody, once a target has been identified, the process of antibody development, though long and costly, is *routine* to one of ordinary skill in the art. Many papers, books, and text books have been published on methods to develop polyclonal or monoclonal antibodies. *See, e.g.,* Liddell & Cyrer, A Practical Guide to Monoclonal Antibodies, John Wiley & Sons, 1st edition, 1991; Goding, Monoclonal Antibodies, Third Edition: Principles and Practice, Academic Press, 1996); Zola, Monoclonal Antibodies: A Manual of Techniques, CRC, 1987. Monoclonal and polyclonal antibody production can be ordered from many companies that offer the service once the antigen is available. *See, e.g.,* http://www.anaspec.com/services/antibody.asp?OVRAW=costume%20monoclonal%20antibody&OVKEY=custom%20monoclonal%20antibody&OVMTC=advanced; http://www.bachem.com/index.cfm?568B69501FB211D6B4CC00500465876C; http:// www.neomps.com/dn_Custom_antipeptide_antibody_service_us/. Nakazato *et al.* teach that anti-ghrelin immunoglobin G robustly suppressed feeding.[13] As mentioned above, an anti-ghrelin antibody is currently being developed in a combined phase I/II clinical trial as a treatment for obesity (*see* http://www.cytos.com/doc/Cytos_Press_050511_E.pdf).

Based on the ghrelin splice variant 2 amino acid sequence, one of ordinary skill in the art can order from a peptide or protein synthesis company ghrelin splice variant 2 and reproduce any of the ghrelin studies, such as the Tschop *et al.* study reproduced by Applicant. One of ordinary skill in the art could, alternatively, use an expression system to produce the claim 31 or 32 invetion (see page 35, line 25 – page 42, line 11 of Applicant's specification). Ghrelin peptides are working in any administration route, and art exists (i.e., the aforementioned 571 papers published prior to Applicant's filing date) from different groups showing the same range of action. Production of anti-ghrelin

[13] Nature 409:194-98 (2001).

splice variant 2 antibodies is a routine exercise for those of ordinary skill in the art. Thus, Applicant respectfully submits that, because only routine experimentation is needed, the claim 31 and 32 inventions are enabled.

Summary

In view of the foregoing amendments and remarks, Applicant submits that this application is in condition for allowance. In order to expedite disposition of this case, the Examiner is invited to contact Applicant's representative at the telephone number below to resolve any remaining issues. Should there be a fee due which is not accounted for, please charge such fee to Deposit Account No. 501447 (Potter Anderson & Corroon LLP).

Respectfully Submitted,

By: _____

Jeffrey B. Safran
Attorney for Applicant
Reg. No.: 54,689
Telephone: (302) 984-6132
Facsimile: (302) 658-1192

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